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New records of *Didymium inconspicuum*, *D. karstensii*, and *D. rugulosporum* from China

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Abstract—*Didymium inconspicuum*, *D. karstensii*, and *D. rugulosporum* are reported as new records for China. Morphological characteristics were observed and described by light microscopy and scanning electron microscopy and are discussed and compared with similar species.

Key words—*Didymiaceae*, *Myxogastrea*, taxonomy

Introduction

Didymium was introduced by Schrader (Schrader 1797) and characterized by sporophores with a peridium covered with stellate lime crystals. In contrast, the capillitium is almost always limeless. The lime crystal nature on the peridium separates *Didymium* from *Diderma*, in which the lime occurs as amorphous granules (Martin & Alexopoulos 1969). Currently *Didymium* includes approximately 94 species around the world (Kirk & al. 2008, Lado 2018), of which 35 have been recorded in China (Li & al. 1996, Liu & Chen 1998, Chen 1999, Li 2008, **Liu & Chang 2011**, Gao & al. 2018). The objectives of this work were to examine some putative *Didymium* samples, characterize them by light and scanning electron microscopy, and compare them with similar species.

Materials & methods

Specimen collection and moist chamber culture

Didymium rugulosporum was collected from Huzhong Nature Reserve, Heilongjiang Province in 2016. *Didymium karstensii* was collected from Changbai Mountain, Jilin Province in 2018. *Didymium inconspicuum* was collected from the desert transition zone of Suwu Township, Minqin County, Gansu Province in 2016. The specimens are deposited in the Herbarium of the Mycological Institute of Jilin Agricultural University, Changchun, China (HMJAU).

Morphological identification

The morphological characteristics of the sporocarps were observed by light microscope and scanning electron microscope. Each sporocarp was placed on a slide and one drop of 4% KOH added. The slide cover was placed on top and gently tapped, and filter paper used to remove excess solution. The capillitium, calcareous crystals, and spores were observed under a Leica DM 2000 light microscope (LM). Up to 20 spores were randomly selected and their diameters measured. The ultrastructure was observed under the Hitachi SU8010 scanning electron microscope (SEM) operating at 5kV (Martin & Alexopoulos 1969, Zhu & al. 2012, Li & al. 2017).

Taxonomy

Didymium inconspicuum Nann.-Bremek. & D.W. Mitch.,
Proc. Kon. Ned. Akad. Wetensch., C 92: 508. 1989 FIG. 1

Sporocarps sessile, scattered, occasionally mingled with short plasmodiocarps, small and inconspicuous. 0.5–1 mm long, about 0.5 mm high, ovoid, often depressed in the center, grayish white. Columella absent. Peridium membranous, thin, coated with grayish white lime crystals. Capillitium profuse, threads slender and curved, pale brown, 1–1.5 µm diam., often branching and anastomosing to an incomplete network, sometimes with flattened and darker colored sections on the branches. Spores dark brown in mass, brown by LM, ovoid to globose, 8–11 µm diam., evenly slightly warted.

SPECIMEN EXAMINED: **CHINA, GANSU PROVINCE, Minqin County**, Suwu Township, 38°16′42″N 103°06′17″E, 1360 m, on leaf by moist chamber culture, 16 September 2016, Chaofeng Yuan & Shuwei Wei (HMJAU 10650).

COMMENTS: *Didymium inconspicuum* is similar to *Didymium obducens* P. Karst. [= *D. fulvum* Sturgis] and *Didymium ochroideum* G. Lister. The spore of *D. obducens* is tuberculate on one side, while the other side is smooth and the color is pale, but the spore of *D. inconspicuum* has relatively uniform small warts. The spore of *D. ochroideum* is 6–8 µm diam. whereas the spore of *D. inconspicuum* is 8–11 µm diam.

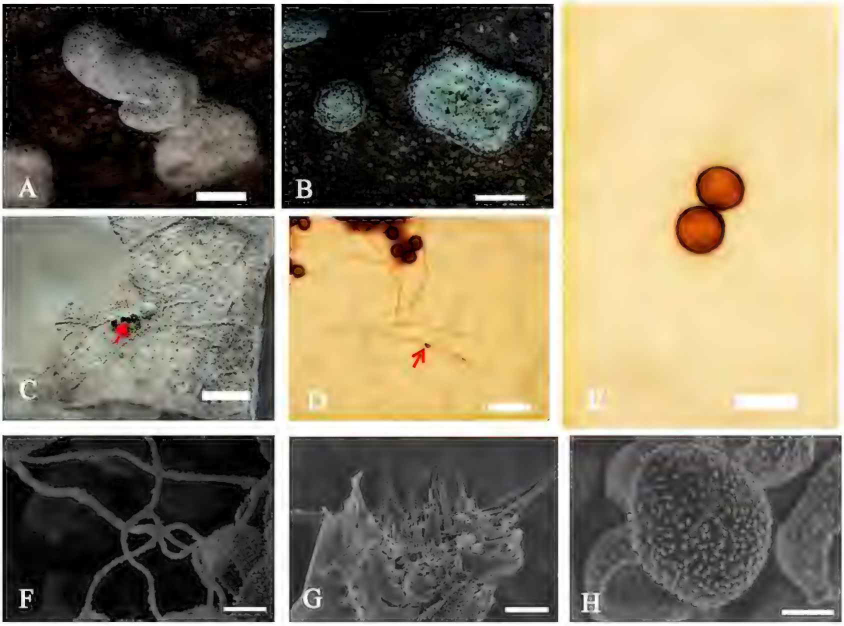

Fig 1. *Didymium inconspicuum.* A– C. Sporocarps; D. Capillitium (LM); E. Spores (LM); F. Capillitium (SEM); G. Lime crystal (SEM); H. Spores (SEM). Scale bars: A– C = 500 µm; D, F = 10 µm; E, G, H = 5 µm.

Nannenga-Bremekamp (1989) described *D. inconspicuum* as having white or pale yellow-brown tiny lime crystals, with a coarse capillitium and spores that are 12–14(–15) µm diam.; Schnittler & Novozhilov (2000) described this species as having 3–6 µm long lime crystals smaller than spores, a pale to pale brown, 1–2(–2.5) µm diam. capillitium, and (9.5–)10.5–11.5(–12.5) µm diam. spores. Our specimen has white lime crystals about 5–8 µm diam., a light brown 1–1.5 µm diam. capillitium, and spores 8–11 µm diam.; other features are consistent with the published descriptions.

Didymium karstensii Nann.-Bremek., Acta Bot. Neerl. 13: 247. 1964 Fig. 2

Sporocarps gregarious, sessile or with a short stalk, white, subglobose, 1 mm wide, up to 0.5 mm high, stalk (when present) calcareous, yellowish white, about 0.2 mm long. Peridium double, outer layer white, with stellate lime crystals about 5 µm diam. forming the wrinkled shell; the inner layer membranous and colorless. Columella white, calcareous, slightly thickened at

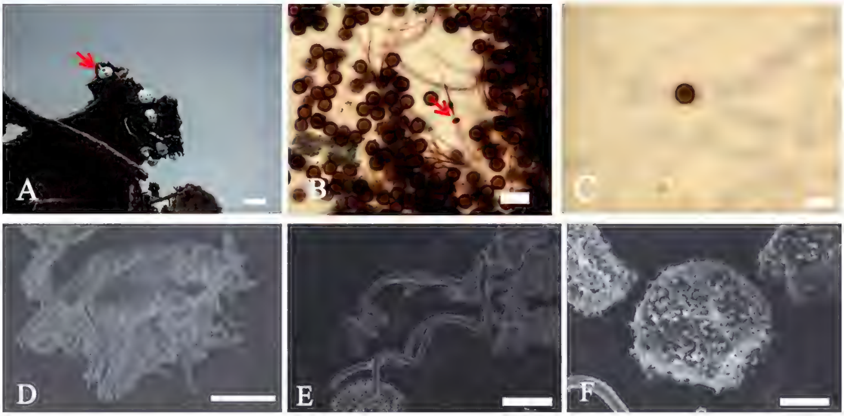

Fig 2. *Didymium karstensii*. A. Sporocarps; B. Capillitium and swelling (LM); C. Spores (LM); D. Lime crystals (SEM); E. Capillitium (SEM); F. Spores (SEM). Scale bars: A = 1 mm; B–E = 10 µm; F = 5 µm.

the base. Capillitium pale brown, threads slender, branching and anastomosing to a network, with dark swellings, 2–3 µm diam., attached to the peridium and to the base of the sporocarp. Spores dark brown in mass, brown by LM, densely warted and ridged, 7.5–10 µm diam.

> Specimen examined: **CHINA, Jilin Province, Changbai Mountain**, 42°22′18″N 128°00′14″E, 828 m, on the leaf, 5 September 2018, Chaofeng Yuan & Shuwei Wei (HMJAU 10651).

Comments: *Didymium karstensii* is similar to *Didymium squamulosum* (Alb. & Schwein.) Fr., but *D. karstensii* is inconspicuously umbilicate at the base, whereas *D. squamulosum* is deeply umbilicate at the base. Nannenga-Bremekamp (1964) described stalks of *D. karstensii* as generally not exceeding half the diameter of the sporocarps, and spinulose spores, 10–12 µm diam. Demirel & Kaşik (2012) described stalks of *D. karstensii* generally not exceeding 0.2 mm long, and warted spores, 10–12 µm diam. Our specimen had stalks about 0.2 mm long and warted spores, 7.5–10 µm across; other features are consistent with the published descriptions.

Didymium rugulosporum Kowalski, Mycologia 61: 636. 1969 Fig. 3

Sporocarps sessile, scattered to clustered, globose to subglobose, 1–2.5 mm diam., white. Peridium double, the outer layer thick and fragile, white smooth calcareous shell, stellate lime crystals; the inner layer membranous, thin, colorless and transparent. Columella absent. Capillitium dense, rigid, brown,

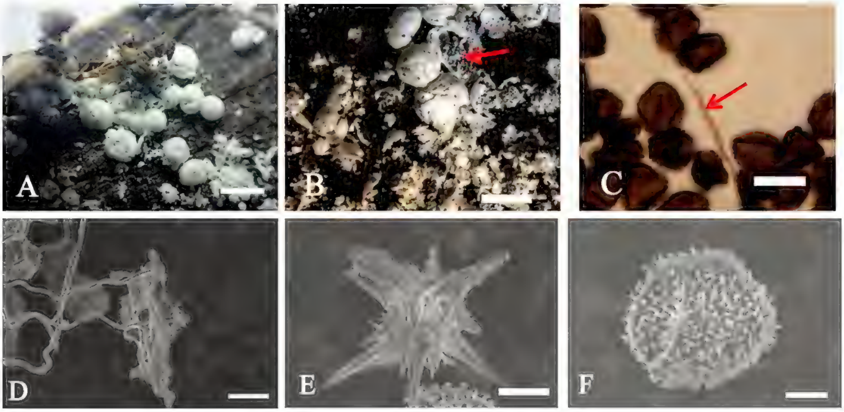

Fig 3. *Didymium rugulosporum*. A, B. Sporocarps; C. Capillitium and spores (LM); D. Capillitium and peridium (SEM); E. Lime crystal (SEM); F. Spores (SEM). Scale bars: A, B = 1 mm; C = 20 µm; D = 10 µm; E, F = 5 µm.

1–1.5 µm diam., weakly attached to the base and apex of the sporocarps, brown, branched with many colorless swellings. Spores dark brown in mass, purple brown by LM, globose, 17–19 µm diam., with large dense warts about 1.5 µm high and with some ridges forming a reticulum.

Specimen examined: **China, Heilongjiang Province, Huzhong Nature Reserve**, 52°06′02″N 123°20′38″E, 168 m, on the bark, 24 September 2016, Wan Wang (HMJAU 10652).

Comments: *Didymium rugulosporum* is similar to *Didymium trachysporum* G. Lister and *Didymium quitense* (Pat.) Torrend, which differ by their smaller spores (*D. trachysporum* 9–10 µm diam.; *D. quitense* is 12–15 µm diameter). Kowalski (1969) described spores of *D. rugulosporum* as (16–)18–20(–22) µm diam. Our specimen had spores 17–19 µm diam.; other characteristics are consistent with the original description.

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